The Epithelial-Mesenchymal Transition, as Hacked by a microRNA Combinatorial Code

EMT HACKED BY A microRNA COMBINATORIAL CODE

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SUMMARY

A new study coupling bioinformatic and experimental investigations highlights the importance of combinatorial microRNA targeting in human EMT, a phenotypic program underlying normal and pathological processes, paving the way for a better understanding of microRNA regulatory networks in humans.

OPENING PARAGRAPH

The vast majority of our genome encodes for non-coding molecules, whose precise functions are mostly unknown. Among these microRNAs (miRNAs), a class of small endogenous regulatory non-coding RNAs, have emerged in the last twenty years as major non-coding RNA species, given the key role they play in the human regulatory network.

The current paradigm is that miRNAs, as a whole, exert their function through a labyrinth of combinatorial interactions, whose complexity however has been up to now only partially explored. In fact, most of the data produced so-far focused their attention on single miRNA-target gene interactions, usually neglecting the intrinsic combinatorial nature of gene regulation in higher eukaryotes and in particular in human, and adopted experimental protocols which require molecular concentration levels far from the physiological ones.

In this respect, a paper by Cursons et al. in this issue of Cell Systems represents a relevant step forward in this direction. Using a combination of bioinformatics, high-throughput sequencing and functional validations, Cursons and colleagues were able to precisely describe the behaviour of a circuit composed by a set of miRNAs acting in a combinatorial way at molecular concentration close to cellular endogenous levels, to drive human Epithelial-Mesenchymal Transition (EMT), an important biological switch implicated both in development and diseases.

MAIN TEXT

MiRNAs are a class of tiny ∼22 nt RNAs whose primary function is to manage post-transcriptional repression of mRNA targets. Pervasive in the human genome, with more than 2.000 examples currently annotated, their primary action is thought to be RNA silencing and translational inhibition of target protein-coding genes (Bartel, 2018).

Both computational as well as experimental clues, support the notion that over half of the human transcriptome is regulated by miRNAs. At the same time, all the miRNA target prediction tools, developed over many years, despite the inherent differences arising from the diversity of the algorithms used, point out that a typical miRNA can accomplish its functions on possibility hundreds of target genes. Conversely, a single gene can be regulated by several miRNAs, suggesting the presence of a complex combinatorial regulatory code embedded in the human post-transcriptional regulatory network (Krek A et al., 2005; Tokar T el al., 2018).

In this context, lot of interest has been attracted in the past years by the study of the interplay between miRNAs and Transcription Factors (TFs) and in particular on their mutual involvement in complex circuits with feed-forward or feedback loop topologies. It has been realized that this class of regulatory motifs have an important role in driving differentiation processes and are involved in various aspects of physiology and disease, including cancer (Bracken CP et al., 2016).

Among these regulatory circuits, one of the most studied is the feedback loop which involves TFs of the Zeb family and miRNAs of the mir-200 family which together control the so called Epithelial to Mesenchymal Transition, a critical step in development and embryogenesis, which has also a major role in tumorigenesis. EMT represents the key process by which differentiated epithelial cells can change their status and gain the ability to invade, to resist apoptosis, and eventually to

disseminate leading to metastasis formation (Gregory PA et al., 2008; Hanahan D and Weinberg RA, 2011).

How do experimentally test the role of miRNAs? A plethora of different wet-lab approaches exist to address this issue, however in most of the cases, classical gain of function or loss of function methods are used, as they represent the simplest way for probing the biological function of molecular species and understand their role in driving biological phenotypes. These approaches proved to be extremely useful for finding operational links between miRNAs and phenotypes and for the identification of direct miRNA targets. However, often the practical realization of these experiments requires supra-physiological miRNA concentrations that may interfere with mRNA transcripts not functionally regulated by the miRNA under study at endogenous expression levels.

The desire to overcome these limitations and investigate the role of combinatorial miRNA action in the context of human EMT, using molecular concentration levels close to physiological ones, has provided the rationale for the study by Cursons and colleagues “Combinatorial targeting by microRNAs coordinates post-transcriptional control of EMT” (Cursons et al., 2018). The authors combined computational and experimental methods to identify co-regulated miRNAs that have the potential to cooperate during EMT and they demonstrate that the combinatorial activity of co-regulated miRNAs is an intrinsic properties of post-transcriptional network regulation. Using a human mammary cell model of EMT, Cursons et al. provide evidence that miRNAs can act as a secondary regulatory layer after transcription, amplifying transcriptional effects on relevant EMT-associated processes (such as cell-adhesion and extracellular matrix organization) whilst simultaneously buffering transcriptional effects on non-EMT genes. They particularly pinpoint that a set of miRNAs, composed by miR-200c-3p, miR-141-3p, miR-182-5p and miR-183-5p, are able to cooperate in a combinatorial manner, through co-regulation and cooperative targeting of functionally related transcripts, even when operating in sub-nanomolar concentrations in the context of EMT.

The main idea of the article (Figure 1) is that co-expressed miRNAs that jointly target multiple genes in a common pathway enhance their common function, a notion the authors addressed also in previous studies (Bracken CP et al., 2016) and is at the core of the present paper. Strikingly, the authors were able to show that co-transfection with low levels of miR-200 and miR-182/183 had a co-operative effect on epithelial gene expression, and an almost negligible effect on off-target mRNAs.

The results of this article indicate that, at least in a specific experimental setup and model, a design based on simultaneous changes in the abundance of several miRNAs is effectively able to produce functional effects with no need of supra-physiological concentrations. The miRNAs exert their action via the collaborative network of several targets, and at the same time, due to the moderate level of concentration do not influence the expression of unrelated genes.

The approach by Cursons and co-workers, goes well beyond the particular EMT pathway to which it has been applied. It points out a new powerful way to investigate the properties of the “real” miRNA post-transcriptional networks (Pinzón N et al., 2017) and could be extrapolated, in principle to other model systems.

In this respect, the use of EMT as model system and the miR-200 / miR-141 axis as a case study, due to the importance of this biological pathway, clearly connote a stunning experimental setup for the investigation proposed (Sass S et al., 2011) but, at the same time, due to the intrinsic complexity of the EMT process, raises the important question whether combinatorial miRNA patterns such the one reported can be identified, and shown to be similarly effective, in other biological conditions. Similar investigation in the future will really help to further address these issues.

Another major reason of interest of this paper is that it could open the way for a fine tuned, reliable and quantitative modelization of miRNA regulation along the line of what was done in the past years for transcriptional regulation (Bintu L et al., 2005). In particular it could allow to address, in physiological conditions, a few long standing issues in miRNA biology like the interplay between the number of miRNA binding sites (and of miRNA species) and their strength or the relevance of the so called “sponge effect” due to the presence of competing endogenous RNA (ceRNA).

One of the major open problems in this context is to understand if it is more effective for the miRNA to bind the target messenger RNA with several weak binding sites or with a single strong one. A complete answer to this question requires not only a careful modelization of miRNA-mRNA interaction but also reliable experimental data in physiological conditions. It would represent a major achievement, which could have relevant implication both from a theoretical point of view and for its potential clinical applications and which seems now within reach thanks to the results of Cursons and co-workers. We hope that future experimental and computational work will substantiate and broaden this design paradigm.

FIGURE LEGEND

Figure 1: *A set of miRNAs acting in a combinatorial manner on a network of target genes coordinates post-transcriptional control of EMT*. Within a cell, co-regulated miRNAs can act in a combinatorial manner on a set of target genes, usually embedded in a network of interactions, eventually interfering with complex phenotypic patterns. Cursons et al. investigated the cooperative role of a combination of few miRNAs, including the miR-200 and miR-182/183 family members, in the post-transcriptional regulation of EMT (human Epithelial-Mesenchymal Transition), an important biological switch implicated both in development and disease. In particular, they proved that the combinatorial treatment could alter the cellular phenotype with miRNA concentrations closer to physiological levels and with less off-target effects.

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